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SO Poultry Science, (1997) Vol. 76, No. 5, pp. 677-682.

SO VETERINARY RECORD, (16 JAN 1993) Vol. 132, No. 3, pp. 56-59.

SO Journal of Parasitology, (1992) Vol. 78, No. 5, pp. 906-909.

SO AVIAN PATHOL, (1986) 15 (2), 271-278.

SO ACTA PARASITOL POL, (1976 (RECD 1977)) 24 (11-19), 103-117.

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Aspergillus flavus.

Avian Pathology, 15: 271–278, 1986

IMMUNISATION OF YOUNG BROILER CHICKENS WITH LOW LEVEL INFECTIONS OF *EIMERIA TENELLA*, *E. ACERVULINA* OR *E. MAXIMA*[1]

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SUMMARY

Chickens given 200 oocysts of *Eimeria tenella* at day-old followed by a dose of between 300 to 500 oocysts at 8 days of age were afforded substantial protection against challenge at 15 and 22 days of age.

Chickens given 2,000 oocysts of *E. acervulina* at day-old were partially protected against challenge infection given at 15 or 22 days of age. When chickens were given doses of 2,000 and 10,000 oocysts at 1 and 8 days, respectively, significant protection against challenge at 15 and 22 days was obtained.

Chickens given five oocysts of *E. maxima* at day-old were partially protected against challenge at 15 or 21 days of age. A dose of 50 oocysts at day-old gave substantial protection judged by body weight changes and lesion scores. The protection was slightly greater when the immunising dose was given at 8 days of age.

The results indicate that with chickens kept on wire floors where the conditions for reinfection were minimal, substantial immunity to challenge infection could be achieved by giving small numbers of oocysts to chickens 1 to 8 days of age.

INTRODUCTION

Chickens maintained under most conditions of poultry management develop clinical or subclinical infections with *Eimeria*. As a consequence, survivors acquire resistance. This is not usually an absolute immunity because most animals continue to discharge oocysts.

Pierce *et al.* (1962) were able to produce absolute immunity to *E. tenella* by

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giving three graded doses (viz. 500, 5,000 and 50,000 oocysts) at weekly intervals starting when chickens were 1 week old. Using two graded doses of *E. acervulina* or *E. maxima*, Rose and Long (1962) demonstrated these species to be more immunogenic than *E. tenella*.

From the practical standpoint, a 'live' vaccine which protects against the pathogenic effects of coccidiosis rather than achieving an absolute immunity is desirable. In this connection, Long and Millard (1977) were able to produce a practical level of immunity by inoculating 1 or 2-week-old chickens with a single dose of oocysts and allowing reinfection to occur under floor-pen conditions. The value of administering small numbers of oocysts per day (5 to 20 oocysts) over a period of 14 to 21 days to establish immunity to *Eimeria* infections in chickens was demonstrated by Joyner and Norton (1973; 1976).

Despite the excellent studies cited above, we are still without a clear idea of the immunising ability of a single small dose of oocysts given to day-old chickens or the effect of giving two small immunising doses in the 1st week of life. Most studies have used chickens 1 to 2 weeks old at the start of the experiment apart from the study with *E. maxima* (Long, 1959) who immunised 3-day-old chickens against *E. maxima* by giving only 500 oocysts.

The objective of the experiments reported here was to determine if chickens kept on wire floors, given oocysts of *E. tenella*, *E. acervulina* or *E. maxima* at 1 day and 8 days of age, developed immunity 7 or 14 days later. The degree of immunity was judged by lesion scores, body weight gain and feed conversion.

MATERIALS AND METHODS

Experimental animals and housing

Broiler chickens (Arbor Acre) were randomly allocated to pens in five Petersime batteries. These batteries have wire floors and each contained 30 pens on five levels. Each treatment group comprised 3 replicate groups of 6 or 8 chickens as indicated in Tables 1 to 3.

Sentinel birds

In order to monitor extraneous infection, sentinel birds were added to six of the treatment groups. Six-day-old sentinel birds, which had been raised in isolation, were introduced into the selected pens when the principal birds were 6 days of age. Three sentinel birds were put into each of the selected pens. In some groups, the sentinel birds were removed at 15 days of age, giving them an in-pen exposure time of 9 days. These sentinel birds were challenged as were the principal birds at 15 days of age.

The remaining sentinel birds were removed at 22 days of age, giving them an in-pen exposure time of 16 days. These sentinel birds were challenged at the same time as the principal birds at 22 days of age.

Source of oocysts

Stock cultures of oocysts are maintained via passage in Hubbard/Arbor Acres broiler chickens at Merck, Sharp & Dohme Research Laboratories. The strains used are standard, drug-sensitive laboratory strains which have been maintained in the laboratory for several years. For experimental infections oocysts of *E. maxima* (Merck strain FS-110), *E. tenella* (Merck strain LS-18) or *E. acervulina* (Merck strain LS-3) were given in 0.5 ml volume orally. Numbers of oocysts used for

Table 1. Immunisation with

Group*	Age of birds (days 1	8	15	22
	Parasites given†			
1	A	B	CH	
2	A	C	CH	
3	A	D	CH	
4	A	B	–	CH
5	A	C	–	CH
6	–	D	–	CH
7	–	A	B	CH
8	–	C	C	CH
9	–	A	D	CH
10	–	B	B	CH
11	–	B	C	CH
12	–	B	D	CH
13	A	–	–	–
14	–	B	–	–
15	–	–	C	–
16	–	–	CH	–
17	–	–	–	–
18	–	–	–	–
19	–	–	–	–

†A = 200 oocysts; B = 300 oocyst
100,000 oocysts.
* 18 birds (3 x 6) per group.
a, b, c, d, e, f Values within colum

inoculation were estimated
diluted to provide approxima

Parameters used to evaluate i
Body weight gain. Chickens
tion, time of challenge infecti

Food conversion. A weighed
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divided by the weight gain
dying during the course of the

Lesion scores. Lesion scores
using the method described b

Data were analysed statistical

Table 1. Immunisation with small numbers of oocysts of E. tenella.

Group*	Age of birds (days) 1	8	15	22	Results 7 days after challenge on days 15 or 22: Avg. wt. gain Day 15	Avg. wt. gain Day 22	Feed conv. Day 15	Feed conv. Day 22	Lesion score Day 15	Lesion score Day 22
	Parasites given†									
1	A	B	CH	–	265[a]		1.740[ab]		1.4[bc]	
2	A	C	CH	–	278[a]		1.698[ab]		1.8[b]	
3	A	D	CH	–	252[a]		1.801[ab]		1.2[c]	
4	A	B	–	CH		345[ab]		1.628[cd]		1.6[cd]
5	A	C	–	CH		370[ab]		1.544[cd]		0.8[e]
6	–	D	–	CH		351[ab]		1.569[cd]		1.8[cd]
7	–	A	B	CH		334[b]		1.736[b]		0.5[b]
8	–	C	C	CH		360[ab]		1.604[cd]		1.2[de]
9	–	A	D	CH		347[ab]		1.630[cd]		1.9[bc]
10	–	B	B	CH		342[ab]		1.614[cd]		1.8[cd]
11	–	B	C	CH		348[ab]		1.632[cd]		2.0[bc]
12	–	B	D	CH		346[ab]		1.621[cd]		2.2[bc]
13	A	–	–	–		389[a]		1.479[d]		0.2[e]
14	–	B	–	–		365[ab]		1.546[cd]		0.0[f]
15	–	–	C	–		346[ab]		1.678[bc]		0.1[f]
16	–	–	CH	–	198[b]		2.091[a]		3.0[a]	
17	–	–	–	–		247[c]		1.960[a]		3.4[a]
18	–	–	–	–	289[a]		2.557[b]		0.0[d]	
19	–	–	–	–		357[ab]		1.583[cd]		0.0[f]

†A = 200 oocysts; B = 300 oocysts; C = 400 oocysts; D = 500 oocysts; CH = Challenge with 100,000 oocysts.

* 18 birds (3 x 6) per group.

a, b, c, d, e, f Values within columns with different superscripts differ significantly ($P<0.05$).

inoculation were estimated from replicate counts using a haemocytometer and diluted to provide approximately the correct number of oocysts.

Parameters used to evaluate immunity

Body weight gain. Chickens were weighed individually at time of oocyst inoculation, time of challenge infection and 7 days after challenge infection.

Food conversion. A weighed quantity of feed was allocated to each pen. At the end of the experiment, the feed remaining was weighed. The weight of this feed was divided by the weight gain of the remaining birds plus the weight gain of birds dying during the course of the experiment.

Lesion scores. Lesion scores were done on all birds 7 days after challenge infection using the method described by Johnson and Reid (1970).

Data were analysed statistically using Duncan's multiple range test.

RESULTS

E. tenella experiment

The design and results are given in Table 1. From the results given in Table 1, it is clear that a substantial degree of protection, judged by body weight gain, lesion scores and feed conversion, was obtained by giving chickens two doses of oocysts at day 1 and day 8 (groups 1 to 5). This protection was seen after challenge on the 15th day (groups 1 to 3). The results were most clear for body weight gain and this protection was significant ($P<0.05$). When the challenge dose was given on the 22nd day (groups 4, 5, 7 to 12), the protection afforded by two doses (day 1 and day 8 or day 8 and day 15) was similar and at a high level, especially judged by

Table 2. Immunisation with small numbers of oocysts of E. acervulina.

	Age of birds (days)				Results 7 days after challenge on days 15 or 22					
	1	8	15	22	Avg. wt. gain		Feed conv.		Lesion score	
Group*	Parasites given†				Day 15	Day 22	Day 15	Day 22	Day 15	Day 22
1	A	B	CH	–	307[a]		1.622[bc]		0.1[a]	
2	A	C	CH	–	312[a]		1.658[bc]		0.0[a]	
3	A	B	·	CH		389[ab]		1.826[a]		0.1[cd]
4**	A	C	–	CH		378[ab]		1.873[a]		0.3[bc]
5**	A	–	–	CH		362[ab]		1.914[a]		0.3[bc]
6	–	A	B	CH		366[ab]		1.830[a]		0.03[d]
7	·	A	C	CH		384[ab]		1.763[a]		0.2[cd]
8**	A	–	CH	–	286[a]		1.781[b]		0.1[a]	
9**	–	A	–	CH		355[b]		1.952[a]		0.07[cd]
10	–	B	–	CH		379[ab]		1.838[a]		0.07[cd]
11	–	C	–	CH		381[ab]		1.809[a]		0.0[d]
12**	A	–	–	–		385[ab]		1.813[a]		0.03[d]
13	–	A	–	–		394[a]		1.732[a]		0.0[d]
14	–	B	–	–		382[ab]		1.871[a]		0.0[d]
15**	–	C	–	–		380[ab]		1.892[a]		0.0[d]
16	–	–	B	–		393[a]		1.783[a]		0.0[d]
17	–	–	C	–		368[ab]		1.914[a]		0.0[d]
18	–	–	–	–	313[a]	384[ab]	1.527[c]	1.862[a]	0.0[a]	0.0[d]
19	–	–	–	CH	201[b]	264[c]	2.211[a]	2.474[b]	3.5[b]	2.8[a]
8 (sentinels)			CH		169		3.270		3.8	
4 (sentinels)				CH		299		2.080		1.3
5 (sentinels)				CH		253		2.270		1.0
9 (sentinels)				CH		282		2.150		1.6
12 (sentinels)				CH		280		2.170		1.3

†A = 2,000 oocysts; B = 10,000 oocysts; C = 50,000 oocysts; CH = Challenge with 2.25 x 10^6 oocysts.

*24 birds (3 x 8) per group. **Sentinel birds added to these pens.

a, b, c, d Values within columns with different superscripts differ significantly ($P<0.05$).

weight gain after challe

E. acervulina experimen

The design and results g

given at day 1 and da

weight and lesion score

what appeared to be a

Table 3. Immunisation

	Age of
	1 8
Group*	Parasi
1	A –
2	B –
3*	C –
4	A –
5	B –
6**	C –
7	– A
8	· B
9*	– C
10	– A
11	– B
12**	– C
13	A A
14	A A
15**	A A
16	A A
17	A –
18	C –
19	– A
20	– C
21	– –
22	– –
23	– –
24	– –
3 (sentinels)	
9 (sentinels)	
15 (sentinels)	
6 (sentinels)	
12 (sentinels)	

†A = 5 oocysts; B = 50 ooc

*24 birds (3 x 8) per group

a, b, c, d, e, f Values within

n the results given in Table 1, it
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ıg chickens two doses of oocysts
ın was seen after challenge on the
ear for body weight gain and this
challenge dose was given on the
fforded by two doses (day 1 and
ı high level, especially judged by

sts of E. acervulina.

after challenge on days 15 or 22			
Feed conv.		Lesion score	
Day 15	Day 22	Day 15	Day 22
1.622^{bc}		0.1^{a}	
1.658^{bc}		0.0^{a}	
	1.826^{a}		0.1^{cd}
	1.873^{a}		0.3^{bc}
	1.914^{a}		0.3^{bc}
	1.830^{a}		0.03^{d}
	1.763^{a}		0.2^{cd}
1.781^{b}		0.1^{a}	
	1.952^{a}		0.07^{cd}
	1.838^{a}		0.07^{cd}
	1.809^{a}		0.0^{d}
	1.813^{a}		0.03^{d}
	1.732^{a}		0.0^{d}
	1.871^{a}		0.0^{d}
	1.892^{a}		0.0^{d}
	1.783^{a}		0.0^{d}
	1.914^{a}		0.0^{d}
1.527^{c}	1.862^{a}	0.0^{a}	0.0^{d}
2.211^{a}	2.474^{b}	3.5^{b}	2.8^{a}
3.270		3.8	
	2.080		1.3
	2.270		1.0
	2.150		1.6
	2.170		1.3

'sts; CH = Challenge with 2.25×10^{6}

these pens.

:s differ significantly ($P<0.05$).

weight gain after challenge.

E. acervulina experiment

The design and results of this experiment are given in Table 2. Two doses of oocysts given at day 1 and day 8 gave significant protection by 15 days judged by body weight and lesion score (groups 1 and 2). A single dose at day 1 (group 5) provided what appeared to be a lesser degree of protection although this was not statistically

Table 3. Immunisation with small numbers of E. maxima.

	Age of birds (days)				Results 7 days after challenge on days 15 or 22					
	1	8	15	22	Avg. wt. gain		Feed conv.		Lesion score	
Group*	Parasites given†				Day 15	Day 22	Day 15	Day 22	Day 15	Day 22
1	A	–	CH	–	246^{c}		1.90^{b}		2.0^{b}	
2	B	–	CH	–	311^{ab}		1.71^{cd}		0.8^{de}	
3*	C	–	CH	–	322^{ab}		1.63^{d}		0.3^{f}	
4	A	–	–	CH		318^{ab}		2.00^{bc}		1.9^{b}
5	B	–	–	CH		289^{b}		2.11^{cd}		1.9^{b}
6**	C	–	–	CH		340^{a}		1.89^{cd}		0.7^{de}
7	–	A	CH	–	293^{ab}		1.72^{cd}		1.3^{c}	
8	–	B	CH	–	325^{ab}		1.59^{d}		0.8^{de}	
9*	–	C	CH	–	328^{ab}		1.68^{cd}		0.4^{ef}	
10	–	A	–	CH		339^{a}		1.90^{cd}		1.4^{c}
11	–	B	–	CH		353^{a}		1.86^{cd}		0.9^{de}
12**	–	C		CH		357^{a}		1.80^{d}		0.6^{e}
13	A	A	CH	–	290^{b}		1.79^{b}		1.0^{cd}	
14	A	A	–	CH	325^{ab}		1.97^{b}		1.3^{c}	
15**	A	A	–	–	332^{a}		1.62^{d}		0.0^{f}	
16	A	A	–	–		352^{a}		1.89^{cd}		0.0^{f}
17	A	–	–	–		357^{a}		1.85^{cd}		0.0^{f}
18	C	–	–	–		370^{a}		1.81^{d}		0.0^{f}
19	–	A	–	–		348^{a}		1.86^{cd}		0.0^{f}
20	–	C	–	–		365^{a}		1.83^{cd}		0.0^{f}
21	–	–	CH	–	156^{d}		2.51^{a}		3.1^{a}	
22	–	–	–	CH	204^{c}		2.76^{a}		3.1^{a}	
23	–	–	–	–	314^{ab}		1.66^{cd}		0.0^{f}	
24	–	–	–	–		341^{a}		1.92^{cd}		0.0^{f}
3 (sentinels)			CH		187		2.43		2.2	
9 (sentinels)			CH		209		2.21		2.3	
15 (sentinels)			CH		109		3.61		3.3	
6 (sentinels)				CH	287		2.02		1.9	
12 (sentinels)				CH	290		1.93		0.9	

†A = 5 oocysts; B = 50 oocysts; C = 500 oocysts; CH = Challenge with 50,000 oocysts.
*24 birds (3 x 8) per group. **Sentinel birds added to these pens.
a, b, c, d, e, f Values within columns with different superscripts differ significantly ($P<0.05$).

different from the level induced by two doses (Table 2). Sentinel birds included to monitor extraneous infection were highly susceptible to challenge (group 8). Similarly, the protection afforded by giving chickens one or two doses of oocysts either at 1 day and 8 days or at 8 days and 15 days was substantial and significant against challenge inoculation at 22 days (groups 3 to 7 and 9 to 11). Although sentinel birds challenged at 22 days were susceptible (groups 4, 5, 9 or 12), they were not as susceptible as the controls (group 19) suggesting some low level accidental infection had occurred.

E. maxima experiment

The design and results of this experiment are given in Table 3. The non-immunised-challenged birds were fully susceptible to challenge at 15 days and at 22 days (groups 21, 22). Immunisation with a single dose of 5 oocysts gave only partial protection, which was slightly better when the dose was given at 8 days rather than at day 1 (groups, 1, 4, 7 and 10). Immunisation with a single dose of 50 oocysts gave substantial protection against challenge, the protection being slightly better when the immunising dose was given at 8 days (groups 8 and 11).

Immunisation with a single dose of five oocysts on day 8 (groups 7 and 10) gave a similar level of protection to immunisation with two doses, one on day 1 and one on day 8 (groups 13 and 14). Both of these approaches gave better immunity than a single dose of five oocysts on day 1 (group 1).

Immunisation with a single dose of 500 oocysts gave excellent protection against challenge, the protection being most complete in birds challenged at 22 days (group 6).

Sentinel birds, birds of the same age which were kept with the birds of groups 3, 6, 9, 12 and 15 were susceptible to challenge at 15 days (groups 3, 9 and 15), but slightly less susceptible at 22 days (groups 6 and 12). This suggests that some recycling of the immunising oocysts had occurred in the sentinels allowing some immunity to develop.

The results of this study show that a substantial degree of immunity can result from giving a single dose of 5 to 500 oocysts of *E. maxima* when chickens are 1 or 8 days of age. The degree of protection is greater when the immunising dose is between 50 to 500 oocysts rather than five oocysts.

DISCUSSION

The results obtained from the experiments reported here show that one or two small doses of oocysts, given first at day-old, stimulates a substantial degree of protection against challenge infection at 15 or 22 days of age. *E. acervulina* was slightly more immunogenic than *E. tenella*. *E. maxima* was the most immunogenic species; some protection being afforded by a dose as low as five oocysts given at 1-day-old. The differences in the immune response of the three species were in accord with the findings of Rose and Long (1962). The immunity demonstrated was not absolute in the case of *E. tenella* or *E. acervulina* but it was remarkably good, affording protection against weight depression, lesion development and adverse food conversion effects. The findings with *E. acervulina* were not in agreement with those of Hein (1968) who found that several thousand oocysts in two doses were needed to obtain immunity. In this connection it is interesting to compare the *E. tenella* results with those obtained by Long *et al.* (1980). These

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5: 79-92.

Immunisation
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workers gave chickens doses of 100 oocysts at 3 and 11 days of age and obtained substantial protection to challenge at 25 days of age.

The immunity demonstrated by challenge of 15 or 22 day old chickens as a result of small doses of oocysts given at 1 and 8 days of age may have been reinforced by some extraneous infection. It is appreciated that preventing reinfection of chickens discharging large numbers of oocysts is extremely difficult. Nevertheless, from the practical standpoint, these studies show that battery-housed chickens in their 1st week of life can be immunised. Similar experiments conducted in floor pens could be expected to provide even greater stimulation of immunity.

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RESUME

Immunisation de jeunes poulets de chair avec des taux faibles d'ookystes d'*Eimeria tenella*, *E. acervulina* ou *E. maxima*

Des poussins d'un jour recevant 200 ookystes d'*Eimeria tenella* puis 300 à 500 ookystes à 8 jours ont été protégés efficacement vis-à-vis d'une épreuve réalisée à 15 et 22 jours d'âge. Des poussins d'un jour recevant 2000 ookystes d'*E. acervulina* ont été partiellement protégés lors de l'épreuve pratiquée à 15 ou 22 jours d'âge. Par contre lorsque les poulets ont reçu à 1 et 8 jours respectivement 2.000 et 10.000 ookystes, une protection significative a été constatée lors de l'épreuve effectuée à 15 et 22 jours d'âge.

Des poussins d'un jour recevant 5 ookystes d'*E. maxima* ont été partiellement protégés lors de l'épreuve faite à 15 et 21 jours. A la dose de 50 ookystes administrés à 1 jour, une protection satisfaisante, basée sur les critères de poids et les lésions, a été observée. La protection a été légèrement supérieure quand la dose immunisante a été administrée à 8 jours d'âge.

Ces résultats indiquent que lorsque des poulets sont maintenus sur du grillage où les conditions de réinfection sont minimes, une immunité substantielle vis-à-vis d'une infection d'épreuve peut être obtenue en administrant un petit nombre d'ookystes à des poulets âgés de 1 à 8 jours.

ZUSAMMENFASSUNG

Immunisation von Broilerküken durch Infektionen mit geringen Mengen von *Eimeria tenella*, *E. acervulina* oder *E. maxima*

Küken, denen am ersten Lebenstag 200 Oocysten *Eimeria tenella* und am 8. Lebenstag eine Dosis zwischen 300 bis 500 Oocysten verabreicht wurden, entwickelten guten Schutz gegen eine Testinfektion am 15. und 22. Lebenstag.

Küken, die am ersten Lebenstag 2000 Oocysten von *E. acervulina* erhielten, waren gegen eine Testinfektion am 15. oder 22. Lebenstag teilweise geschützt. Wurden den Tieren am ersten und achten Lebenstag 2000 bzw. 10000 Oocysten verabreicht, so wurde ein signifikanter Schutz gegen eine Testinfektion am 15. und 22. Lebenstag erzielt.

Küken, die fünf Oocysten von *E. maxima* am ersten Lebenstag empfingen, waren gegen eine Testinfektion am 15. oder 21. Lebenstag teilweise geschützt. Eine Dosis von 50 Oocysten am ersten Tag führte zu einem kräftigen Schutz, beurteilt auf Grund des Körpergewichtes und der pathologischen Veränderungen. Der Schutz war noch etwas besser, wenn die immunisierende Dosis im Alter von 8 Tagen verabreicht wurde.

Die Ergebnisse besagen, daß bei Küken, bei denen die Bedingungen für eine Reinfektion wegen der Haltung auf Drahtfußböden sehr ungünstig sind, eine kräftige Immunität gegen eine Testinfektion durch Verabreichung kleiner Oocytenmengen im Alter von ein bis acht Tagen erreicht werden kann.

RESUMEN

Inmunizacion de pollos de engorda jovenes con bajos niveles infecciosos de *Eimeria renella*, *E. acervulina* o *E. maxima*

Se logró conferir una protección sustancial a pollitos de un día de edad, a los cuales se les dio 200 ooquistes de *Eimeria tenella* al día de vida, seguida de otra dosis de 300 a 500 ooquistes a los 8 días de edad, habiéndose desafiado a los 15 y 22 días de vida.

Pollos a los cuales se les dio 2000 ooquistes de *E. acervulina* al día de edad, fueron parcialmente protegidos contra desafíos hechos a los 15 y 22 días de vida. Cuando a dichos animales se les administraron dosis de 2000 y 10000 ooquistes a 1 día y 8 días respectivamente, se obtuvo una protección importante contra desafíos hechos a los 15 y 22 días.

Pollos a los cuales se les inocularon cinco ooquistes de *E. maxima* a un día de edad, fueron parcialmente protegidos contra el desafío a los 15 y 21 días de edad. Una dosis de 50 ooquistes a un día de vida confirió una protección sustancial tomando en cuenta los cambios en el peso corporal y el grado de las lesiones. La protección fue ligeramente mayor cuando la dosis inmunizante fue dada a los 8 días de edad.

Los resultados indican que las aves criadas en pisos de alambre bajo condiciones de reinfestación mínimas, una inmunidad sustancial contra desafíos puede ser alcanzada por medio de la administración de pequeñas cantidades de ooquistes a pollos de 1 a 8 días de edad.

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